with the following parameter changes: Sample volume 40 μL; Diluent volume 14 μL; Calculation mode 0. When modified as above, results are reported in milligrams per liter and cover the range 1.7–54.5 mg of albumin per liter.

We assessed the precision of this method by multiple analyses of serum (n = 24), CSF (n = 25), and urine (n = 25) containing 43 g/L, 130 mg/L, and 7.5 mg/L, respectively. Respective intra-assay CVs were 2.9, 1.95, and 4.3%.

Ten urine samples from diabetic children were assayed by the Cobas-Bio and Behring Laser Nephelometer methods. The regression equation relating the Cobas-Bio (x) and nephelometric (y) results was: \( y = 1.07x - 0.17 \) (r = 0.998). Albumin excretion by nine healthy adults was 3.2 to 13.3 mg/L, measured in a daytime specimen. This fast, inexpensive procedure avoids problems associated with use of calibration solutions having a low concentration of albumin.

Reference

A Quality-Control System for the "Activated Clotting Time" Test, Frank A. Sedor, Elizabeth Mayo, and Kathryn E. Kirwan (Hospital Laboratories, Dept. of Pathol, Duke Univ. Med. Center, Durham, NC)

"Activated clotting time" (ACT) is used to monitor heparinization in hemodialysis and cardiopulmonary-bypass surgery, and before arteriography. Rapid (<10 min) and convenient, it can be performed at the bedside and is recommended for monitoring heparinization for patients with no underlying coagulation problems (1, 2). In our hospital, the assay is performed adjacent to the patient, regardless of clinical service, with use of the "Hemochron 400 Heparin Monitoring System" (International Technidyne, Edison, NJ 08820). Arterial blood (2.0 mL) is transferred by air-tight syringe to a Vacutainer Tube containing distomaceous earth activator and a magnetic stirbar. The timer is started and the tube is inserted into the instrument, activating a motor to drive the stirrer. The act of clotting stops the magnet, triggering an alarm and stopping the timer to mark the ACT.

The laboratory must provide a measure of the reliability of the system. A whole-blood quality-control system is the most desirable but least feasible. Alternatively, the manufacturer suggests a procedure involving activated partial thromboplastin time (APTT) controls at two levels. This procedure requires three pipetting and incubation steps to yield ACTs of approximately 60 and 180 s. This is compared to a target ACT of 400 s for cardiopulmonary-bypass surgery (2), 180 to 240 s for prolonged extracorporeal circulation (3).

We devised a system based on use of outdated fresh frozen plasma (FFP). Titration of 2.0-mL aliquots of FFP with conveniently available CaCl₂ solution (2.72 mol/L, injectable) demonstrated a nonlinear positive clotting response to concentration of calcium. For convenience of preparation and reasonable clotting times, we used 25 μL of the CaCl₂ solution (65 μmol CaCl₂), as follows: (a) Transfer 25 μL of CaCl₂ solution to individual ACT tubes and refrigerate. (b) Mix units of outdated FFP well, and transfer 2.5-mL aliquots into tubes and maintain at −20 °C. As needed, thaw the FFP fractions at room temperature. Initiate the test by pipetting 2.0 mL of FFP into the calcium-adulterated ACT tube, and treat it as if it were a patient's sample.

The precision of the FFP and APTT systems is tabulated below:

<table>
<thead>
<tr>
<th></th>
<th>FFP</th>
<th>APTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \bar{x} )</td>
<td>CV, %</td>
</tr>
<tr>
<td>Within day (n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>311</td>
<td>6.1</td>
<td>181</td>
</tr>
<tr>
<td>Day to day (n = 20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>347</td>
<td>6.8</td>
<td>192</td>
</tr>
</tbody>
</table>

Each batch of FFP is usable for at least 21 days. ACT has averaged 261 s during the 18 months the FFP quality-control system has been used. Note that this system addresses only the operation of the instrument. Compared with the commercial APTT reagent, we find the FFP similar in quality as well as economical and convenient.

References

Re-Use of Random Access Fluid Recovered from the Waste of the Technicon RA-1000, Stephen P. Harrison (Dept. of Biochem., Bradford Royal Infirmary, Duckworth Lane, Bradford, BD9 6RJ, U.K.)

"Random Access Fluid" (RAF), a polyfluorinated hydrocarbon liquid, is used in the RA-1000 to eliminate carryover between samples and reagents. We use about 4 L per annum and, at £0.50 per milliliter, this contributes significantly to the running costs for the instrument. Because RAF is an inert material, immiscible with water (1), most of it may easily be recovered and reprocessed, to be used again. RAF is deposited at four sites in the RA-1000: in the reaction tray, in the reagent boats, in the sample- and reagent-probe reservoirs, and in the sample cups. RAF deposited in the sample cups is potentially dangerous to recover, but the waste from the other three sites may be collected into a suitable wide-mouthed container half filled with a sterilizing 50 g/L solution of sodium hypochlorite and reprocessed, conveniently at monthly intervals, as described below.

The waste liquid collected consists of two phases: the top aqueous layer and the lower RAF-reagent precipitate-water emulsion layer. Most of the aqueous layer is removed by pouring off and the lower layer is washed repeatedly with water, pouring off after each addition, until most of the reagent precipitate is removed. The unrefined RAF is transferred to a separate funnel, where the lower RAF emulsion is removed, leaving any remaining reagent precipitate in the funnel. The RAF is further sterilized by mixing with an equal volume of 50 g/L sodium hypochlorite solution. The layers are allowed to separate and the upper aqueous layer is removed. The RAF emulsion is now washed in a similar manner with two equal volumes of distilled water. After the final wash as much as possible of the top (aqueous) layer is removed and the emulsion is dehydrated by adding anhydrous sodium sulfate (about 1 g per 5 mL of RAF). The
purified RAF, after being filtered through Whatman No. 1 filter paper, is ready for use in the RA-1000.

Comparison of recycled RAF with unused RAF for 30 patients’ sera showed no significant difference (paired t <1.5, P > 0.1) for the following assays: Na⁺, K⁺, HCO₃⁻, Cl⁻, urea, creatinine, C₆H₅OH, albumin, M₆H₂O₄, and amylase. Recycled RAF has been supplementing unused RAF in our system for nine months with no deterioration in instrument performance. We estimate a current savings of approximately £1000 per annum on our present consumption.

Reference


We have developed a microcomputer program, written in BASIC, for statistical evaluation of comparison of methods, which may be applicable to the NCCLS evaluation protocol EP9-P and the 2nd Draft Guidelines for Evaluation of Analyzers in Clinical Chemistry of the ECCLS (1, 2). BASIC as language will be accessible, because application of microcomputers for BASIC use is widespread, even in small laboratories.

The BASIC program needs 64-kbytes memory, a disc drive, and a plotter. As hardware we use a HP85-3 microcomputer with a random access memory of 64 kbytes, including a thermal printer, a micro disc drive HP-9121D, one flexible micro disc (3.5 inch) HP-92191A having a storing capacity of 270 kbytes, and a graphic plotter HP-7470A (Hewlett-Packard Co, Corvallis, OR).

Duplicate results obtained from specimens with the test method and the comparative method may be used. Specimen capacity is up to 250. During input of results, the results are printed, as are the absolute difference of the x and the absolute difference of the y duplicates, and a sequence number per specimen is added. If any single duplicate exhibits an absolute difference exceeding four times the mean of the absolute differences for that method, the duplicate is identified, and an additional calculation is made. The relative absolute difference of the identified duplicate is calculated. If this single duplicate exhibits a relative absolute difference of four times the mean relative absolute difference, that specimen is deleted from the data.

A scatterplot of y vs x is made, with the test method as y-axis and the comparative method as x-axis. A dotted line y = x may be added. Total number of specimens, range of x values and range of y values, mean of all x values and mean of all y values, SD mean x values and SD mean y values, slope, SD slope, intercept, SD intercept, coefficient of correlation, standard error of estimate, and Deming Sₓₓ are printed. Formulas for calculating slope and intercept of an orthogonal regression line and the standard error of estimate are used from Cornbleet and Gochman (3). The formulas for calculation of the standard deviation of the slope and the standard deviation of the intercept are based on Patefield (4). A coefficient of correlation is used as guide to assess if the range of data is sufficiently wide.

Two ways to detect outliers from the calculated regression line can be used. The first: absolute residuals yᵢ - xᵢ exceeding four times the standard error of the absolute residuals are excluded from a second calculation of the orthogonal regression line (5).

The second: if absolute residuals yᵢ - xᵢ exceeds four times the mean of the absolute residuals, an additional calculation is made. For such data set xᵢ,yᵢ the relative absolute residual should not exceed four times the mean of the relative absolute residuals. A single data set found as an outlier may be deleted (1).

A second plot is made with the residuals yᵢ - xᵢ plotted against the xᵢ values. This plot can be used to assess the source of eventual non-linearity. According to Burnett (5) a second-degree polynomial of the form y = a₀ + bₓ + bₓ² is calculated to test for linearity. Subsequently the obtained value of bₓ is to be tested if it differs significantly from zero with Student's t-test value as calculated by the program.

Next the program breaks up the data into three groups of approximately equal size, based on the mean duplicate concentrations of the specimen from the comparative method, so groups of low, middle, and high are formed. For each group a precision is calculated from the pairs of duplicate observations on both the test and comparative methods. The calculated precision gives information on whether the precision remains reasonably equal at different concentrations.

A copy of this program with an example of hard copy is available from the authors or the Editorial Office.

References
2. European Committee for Clinical Laboratory Standards. 2nd draft guidelines for the evaluation of analyzers in clinical chemistry. ECCLS Document Vol. 4, No. 1, January 1984.

Simple and Rapid Liquid-Chromatographic Method for Simultaneous Measurement of Vanillylmandelic Acid and Homovanillic Acid, C. N. Ong; B. L. Lee; H. Y. Ong; and E. Jaecobó (1 Dept. of Community Medicine, Natl. Univ. of Singapore, Singapore 0511; 2 Clin. Biochem. Labs., Singapore General Hospital)

4-Hydroxy-3-methoxymandelic acid (VMA) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) in urine can be determined by several techniques, including the classical spectrophotometric method or gas chromatography or, more recently, by "high-performance" liquid chromatography (HPLC) with ultraviolet or electrochemical detection (Clin Chem 1983;29:876–8). However, most of these techniques require extensive treatment of urine and others are too complicated for routine analysis.

We report here a rapid procedure for simultaneous determination of VMA and HVA, simple enough for analysis of multiple samples. Percolate 2 mL of urine sample (adjusted to pH 6) through an ion-exchange column containing 500 mg of Dowex-1; discard the effluent. Wash the column twice with 2 mL of phosporic acid (10 g/L), then with 2 mL of deionized water, and discard both washes. Elute VMA and